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Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*)

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The Hamilton and Zuk hypothesis on haemoparasite-mediated sexual selection and certain studies of reproductive costs are based on the assumption that avian blood parasite infections are detrimental to their hosts. However, there is no experimental evidence demonstrating harmful effects of blood parasites on fitness in wild populations, it even having been suggested that they may be non-pathogenic. Only an experimental manipulation of natural blood parasite loads may reveal their harmful effects. In this field experiment we reduced through medication the intensity of infection by *Haemoproteus majoris* and the prevalence of infection by *Leucocytozoon majoris* in blue tits (*Parus caeruleus*), and demonstrated detrimental effects of natural levels of infection by these common parasite species on host reproductive success and condition. The fact that some of the costs of infection were paid by offspring indicates that blood parasites reduce parental working capacity while feeding nestlings. Medicated females may be able to devote more resources to parental care through being released from the drain imposed upon them by parasites and/or through a reduced allocation to an immune response. Therefore, this work adds support to previous findings relating hosts' life-history traits and haematozoan infections.

Keywords: *Haemoproteus*; *Leucocytozoon*; primaquine; *Parus caeruleus*; reproductive costs

1. INTRODUCTION

Since the seminal paper by Hamilton & Zuk (1982) proposing that haematozoa may drive sexual selection in birds, many studies have searched for effects of these parasites on mate choice and the evolution of sexual ornaments (Read 1991; Zuk 1992). Their theory required parasites that debilitate their host rather than either kill it or allow total recovery after brief sickness (i.e. chronic and pathogenic). Also, infection by haematozoa has been considered as a potential cost of reproduction and a possibly crucial factor in the evolution of avian life histories (Allander 1997; Møller 1997; Ots & Horak 1998; Wiehn *et al*. 1999). However, all these studies are based on correlations between prevalence or intensities of infection and several hosts' traits. There is a lack of experimental results supporting pathogenic effects in wild populations, probably due to the difficulty of manipulating parasite loads in the field. Clear detrimental effects on host fitness have been reported from poultry (Atkinson *et al.* 1988; Morii 1992), where some of these parasites produce high economic losses (Morii 1992). Studies on captive birds (Earlé *et al.* 1993; Graczyk *et al.* 1994) or endemic island avifauna exposed to novel haematozoa (Warner 1968) also show that infections may induce high mortality rates. Blood parasites normally provoke chronic infections in the wild with relapses during stressful situations for the hosts (Atkinson & Van Riper 1991; Bennett *et al.* 1993), especially during breeding (Weatherhead & Bennett 1991). The capacity of hosts to maintain infections below the threshold above which their effects become apparent makes it difficult to show their pathogenicity in wild birds (Atkinson & Van Riper 1991; Weatherhead & Bennett 1991). In fact, both haemoproteids (Fallis & Desser 1977) and trypanosomes (Baker 1976) have been considered as generally non-pathogenic. The best way to really test the assumption of pathogenicity is to reduce natural infection levels in the wild and look for effects on host fitness traits like reproductive success or condition-dependent survival. In the present field study we experimentally reduced, through medication, the infection intensities and prevalence of two common blood parasite species (*Haemoproteus majoris* and *Leucocytozoon majoris*) naturally infecting breeding females of a wild population of blue tits (*Parus caeruleus*). We demonstrate that there exists a cost of parasitism that is paid in terms of reduction in reproductive success and post-breeding condition.

2. METHOD

The high prevalence of infection by several species of haematozoa in a wild population of blue tits breeding in central Spain (Fargallo & Merino 2000) allowed testing for the effect of reduction of parasitization on host reproductive success.

In 1999, blue tit nests were paired according to hatching date (± 1 d) and brood size (33 pairs of broods were established) and randomly assigned to one of two treatments.

Female parents were captured when their nestlings were three days of age (hatching date = age 0) with traps mounted on nest-boxes, and injected subcutaneously with either 0.01 mg of primaquine (Sigma, St Louis, MO, USA) in 0.1 ml of saline solution or the same quantity of pure saline solution. Primaquine is a chemical compound with antimalarial effects when used at 10 mg kg^{-1} (Graczyk *et al.* 1994; Mayorga *et al.* 1997*a*). As do most antimalarial drugs, primaquine shows non-desirable side-effects, such as gastrointestinal disturbances and development of methaemoglobinaemia and haemolytic anaemia, which are dose dependent (Mayorga *et al.* 1997*b*). Thus, we reduced treatment

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to a low-concentration single dose to minimize these effects. In any case, the toxicity of primaquine rules out the possibility that any beneficial side-effects of medication, other than reduction in blood parasitization, occur.

At this capture, but before treatment, a blood sample was taken from the brachial vein and immediately smeared and air-dried to check for the presence of haematozoan infections. At this capture the birds were also weighed (two escaped after injection but before weighing) and individually identified with numbered metal rings.

When nestlings reached 13 days old we recaptured as many female parents as possible (some became trap-shy), took a second blood sample to check for the effect of treatment on parasite intensities and prevalence, and re-weighed them.

Laying date, clutch size, hatching date and brood size at hatching were obtained by inspecting nest-boxes regularly (day 1 = 1 April).

Tarsus length of all nestlings was measured at 13 days of age (blue tit chicks fledge at 17 days of age in the study population) with a dial calliper to the nearest 0.01 mm and they were weighed with a Pesola (Baar, Switzerland) spring balance to the nearest 0.1 g. Paired *t*-tests were used for measures of reproductive success, as conditions of normality and homoscedasticity were fulfilled. Fledging success was subjected to arcsine square-root transformation before parametric statistical analyses.

Blood samples were fixed in absolute ethanol, and stained with Giemsa stain for 45 min. Half a smear was scanned at 200 in search of large parasites such as *Trypanosoma* and *Leucocytozoon*, whereas small intra-erythrocytic parasites, such as *Haemoproteus* were detected with oil immersion at 1000 magnification. The intensity of infection by *Haemoproteus* was obtained as the number of parasites per 2000 erythrocytes (Merino & Potti 1995; Merino *et al.* 1997). The low number of *Leucocytozoon* and *Trypanosoma* makes it difficult to obtain intensities of infection, so we have used a presence–absence index for analyses of these parasites. *Hepatozoon*, *Plasmodium* and microfilaria have not been considered in statistical analyses given their extremely low prevalences. We predicted that medicated females should show reduced intensities and/or prevalences of infection with respect to control females. Therefore when testing these predictions we have used one-tailed probabilities.

3. RESULTS

At the beginning of the nestling period 87.9% of females ($n = 66$) were infected, as shown by blood smears. The more common blood parasite was *Haemoproteus* (76.9%), followed by *Leucocytozoon* (35.4%) and *Trypanosoma avium* (10.8%). Only two females appeared to be infected by *Hepatozoon parus*, one by microfilaria and one by *Plasmodium* sp.

Before treatment, there were no significant differences between females subsequently injected with the antimalarial drug and those injected with saline either in the intensity of infection by *Haemoproteus* (Mann–Whitney *U*-test, $Z = 0.28$, $p = 0.78$; figure 1*a*) or in the presence of infections by *Leucocytozoon* and *Trypanosoma* ($\chi^2_1 = 0.37$, $p = 0.44$ (figure 1*b*), and $\chi^2_1 = 0.00$, $p = 1.00$, respectively).

However, as expected, medicated females experienced a reduction in the intensity of infection by *Haemoproteus* (Wilcoxon matched-pairs test, $Z = 2.18$, $p = 0.015$), but not females injected with saline solution ($Z = 0.00$, $p = 0.50$, figure 1*a*). Moreover, there was a significant difference in final *Haemoproteus* infection intensity between treatments by introducing initial intensity as covariate in an ANCOVA ($F_{1,58} = 4.45$, $p = 0.039$). In addition, the prevalence of *Leucocytozoon* increased significantly in the control group and decreased significantly in the medicated group (figure 1*b*). There were significantly more birds changing status from uninfected to infected by *Leucocytozoon* than birds recovering from infection in the control group (McNemar $\chi^2_1 = 3.27$, $p = 0.035$). Also, there was a tendency to change status from infected to uninfected by *Leucocytozoon* in the medicated group (McNemar $\chi^2_1 = 1.45$, $p = 0.114$). Moreover, more females maintained the uninfected status than the infected status in the medicated group, but no difference appeared in the control group (McNemar $\chi^2_1 = 5.06$, $p = 0.012$, and $\chi^2_1 = 0.21$, $p = 0.323$, respectively). No apparent treatment effect on *T. avium* was detected (McNemar χ^2, $p > 0.3$ for birds changing status in both groups, and $p < 0.001$ for birds maintaining uninfected status in both groups).

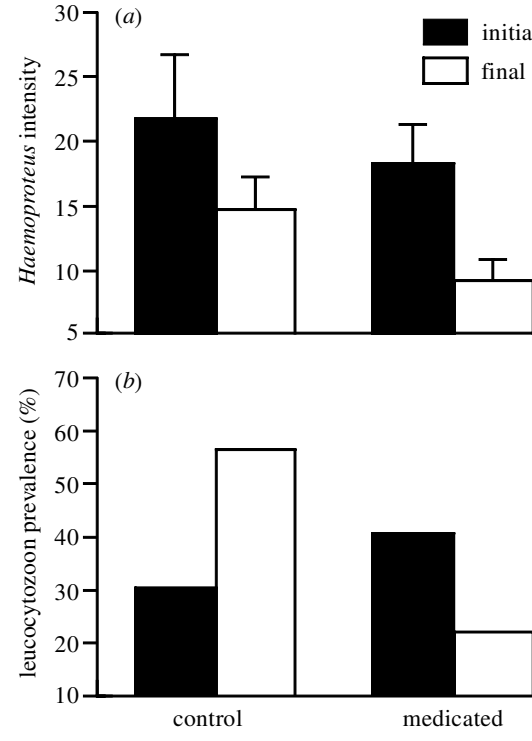

Figure 1. Comparison between initial and final infections in control and medicated female blue tits. (*a*) Mean intensity of infection (s.e.) by *Haemoproteus*. (*b*) Prevalence of *Leucocytozoon*.

Fledging success (percentage of hatched young that reached 13 days of age) was significantly lower for control females (table 1). The proportion of nests with nestling mortality was also significantly higher in the control group (30.3% versus 6.1%, $\chi^2_1 = 4.99$, $p = 0.023$). Body mass and tarsus length of nestlings at 13 days of age and female body mass at this stage did not differ significantly between treatments (table 1). However, there was a significantly negative correlation between the final infection intensity by *Haemoproteus* and final female body mass in the control group (figure 2*a*), but not in the medicated group (figure 2*b*). The body mass of females in both treatments at first capture did not differ ($t = 0.17$, $p = 0.87$).

Table 1. *Average brood size (s.e.) and reproductive traits at 13 days of age of nestlings from medicated and control female parents and the mass of their female parents at this time*
(All tests include 33 pairs of nests, except for final female body mass ($n = 26$).)

	medicated	control	paired *t*-test	*p*
brood size at hatching	7.36 (0.35)	8.03 (0.26)	1.58	0.123
fledging success (%)	98.03 (1.41)	91.90 (3.33)	2.09	0.044
fledgling mass (g)	10.33 (0.15)	10.12 (0.15)	0.94	0.353
fledgling tarsus (mm)	15.99 (0.09)	16.04 (0.13)	0.40	0.690
final female parent mass (g)	10.41 (0.09)	10.36 (0.09)	0.32	0.751

4. DISCUSSION

To our knowledge, this is the first study showing experimentally a detrimental effect of blood parasites on reproductive success and parental condition in a wild-bird population. A clear effect of a reduction in *Haemoproteus* infection intensity and *Leucocytozoon* prevalence on blue tit fitness appeared, as females in the control group suffered a reduction in fledging success compared with primaquine-treated females. There was also a parasitization-dependent deterioration in condition in control females which was not apparent in medicated females, probably due to the absence of high intensities of infection among these females (figure 2). The fact that some clear effects on breeding success and female condition emerged with only a partial reduction in parasitization, indicates that greater deleterious effects of blood parasites than detected by us may occur in their hosts. In addition, it is important to note that we are reporting effects on hosts by two common blood parasites, as the most frequently encountered haematozoan parasites infecting birds in the world are species of *Haemoproteus* (67% of birds sampled; Bennett *et al.* 1982) and species of *Leucocytozoon* are the most prevalent in birds from central Spain (39%; Merino *et al.* 1997). Studies from domestic or aviary-reared birds have shown that infections by *Leucocytozoon* and *Haemoproteus* may cause pathogenic alterations in a wide range of tissues. Myositis appears as the main cause of mortality in these species (Atkinson *et al.* 1988; Morii 1992). However, the special rearing conditions of domestic poultry make it difficult to extrapolate the situation to wild birds. Field studies which have not manipulated infection by haematozoa have reported relationships between parasitization and life-history traits in birds (Møller 1997), but failed to show a clear causal effect between both factors, as they may be influenced by a third undetected element. Only experimental reductions of parasite loads may reveal the causality in the association of natural infection levels with life-history variables.

The fact that some of the effects of infections in female blue tits were paid for by nestlings, implies that the level of parental effort by females is limited by infections, with an increase in effort inducing an increase in infection risk (Ots & Horak 1998; Wiehn *et al.* 1999). This may, in turn, have detrimental consequences for their future reproductive prospects. The apparent paradox that more blood parasites are associated with both lower (this study) and higher (Ots & Horak 1998; Wiehn *et al.* 1999) effort levels, depending on whether effort or parasites are manipulated, may be resolved by the postulated trade-off between reproductive effort and immunity (Sheldon & Verhulst 1996). Medicated females may be able to increase the amount of resources devoted to parental care through being released from the drain imposed upon them by parasites and/or through a reduced allocation to immune responses (Sheldon & Verhulst 1996). The amount of energy expended by females in parental care is known to positively affect the growth and health state of nestlings in another hole-nesting passerine (Merino *et al.* 1996; Moreno *et al.* 1997).

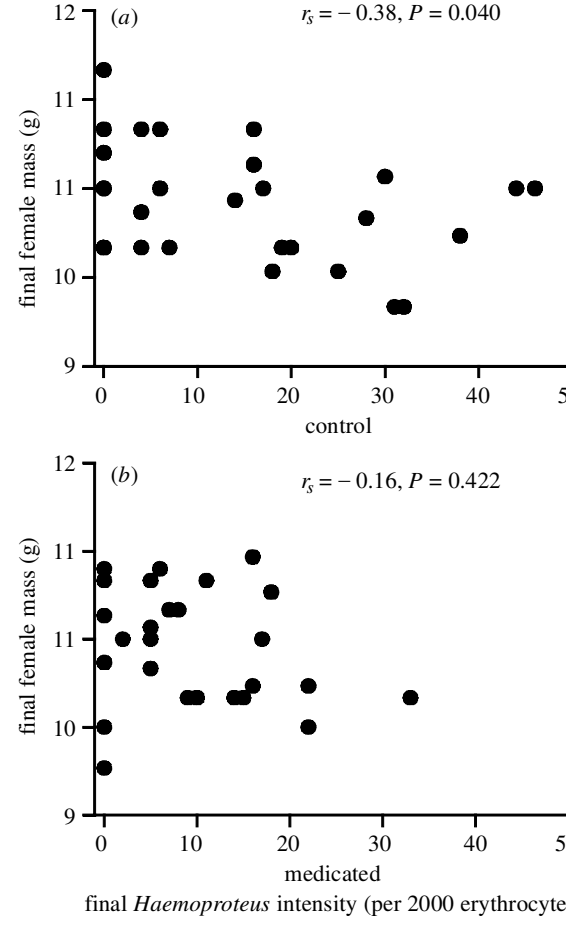

Figure 2. Correlation between the intensity of infection by *Haemoproteus* and female parent body mass when their nestlings were 13 days old. (*a*) Control group ($rs = -0.38$, $n = 31$, $p = 0.040$). (*b*) Medicated group ($rs = -0.16$, $n = 28$, $p = 0.422$).

The hypothesized effects of haematozoa on host sexual selection proposed by Hamilton & Zuk (1982) and in the avian life-history literature (Møller 1997) are based on the assumption of detrimental effects caused by blood parasites on host fitness. This assumption is here validated experimentally by the deleterious effects of natural levels of infection by two common and widely distributed blood parasites on fledging success and female condition in a wild-bird population.

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